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ning of each regular issue of the PCT Gazette.

(54) Title: LABELLING AGENTS FOR MASS SPECTROMETRY COMPRISING TERTIARY AMINES

(57) Abstract: Provided is a method for characterising a molecule by mass spectrometry, which molecule comprises one or more free amino groups, which method comprises: (a) reacting one or more free amino groups in the molecule with a mass tag reagent comprising a reactive functionality capable of reacting with an amino group, and a tertiary amino group linked to the reactive functionality; and (b) characterising the molecule by mass spectrometry.

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## INTERNATIONAL SEARCH REPORT

International Application No  
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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/58 G01N33/68 C07K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HARALAMBIDOU, E. &amp; DAY, R.A.: "Effect of positional isomerism on peptide fragmentation. A comparison of dimethylaminobenzylidene and benzoyl derivatives"</p> <p>ORGANIC MASS SPECTROMETRY, vol. 10, 1975, pages 683-697, XP009032736</p> <p>NORTHERN IRELAND abstract; page 684, second and third paragraphs; figures 1-3; page 696, second paragraph</p> <p>-----</p> <p>-/--</p>	<p>1-13, 15-39</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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## INTERNATIONAL SEARCH REPORT

International Application No  
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DAY R A ET AL: "N-terminal groups in mass spectrometry of peptides. A study including some new and useful derivatives."</p> <p>THE JOURNAL OF ORGANIC CHEMISTRY. 23 FEB 1973, vol. 38, no. 4, 23 February 1973 (1973-02-23), pages 782-788, XP002286140 ISSN: 0022-3263 abstract; figures 1-9; table I</p>	1-13, 15-39
Y	<p>US 2003/044864 A1 (LEVIN MICHAEL ET AL) 6 March 2003 (2003-03-06)</p> <p>claims 1,11,17,43,50-53,59,60</p>	15-17, 19-22, 33-39
Y	<p>WO 02/066988 A (BATTELLE MEMORIAL INSTITUTE) 29 August 2002 (2002-08-29)</p> <p>page 14, lines 3-7; page 15, lines 25-32; paragraph joining pages 16 and 17; claims 1,4,9</p>	15-17, 19-22, 33-39
Y	<p>US 5 281 698 A (NITECKI DANUTE E) 25 January 1994 (1994-01-25) column 2, line 39 - line 41; figure 2</p>	1-13, 15-39
A	<p>LEWIS M R ET AL: "A facile, water-soluble method for modification of proteins with DOTA. Use of elevated temperature and optimized pH to achieve high specific activity and high chelate stability in radiolabeled immunoconjugates"</p> <p>BIOCONJUGATE CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 5, no. 6, 1994, pages 565-576, XP002138319 ISSN: 1043-1802 abstract; page 566, left-hand column, fourth paragraph; scheme 1; page 569, left-hand column, lines 18-21</p>	

# INTERNATIONAL SEARCH REPORT

national application No.  
PCT/GB2004/001167

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-13, 15-39 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-13, 15-39 (all partially)

Mass tag reagents having esters of carbonic acids as the amino-reactive functionalities, their kits and uses for the characterization of molecules by mass spectroscopy and/or for molecule purification. Claims 1-39 are partially to be considered within this group in so far as the reactive functionalities of the mass tag reagents are esters of carbonic acids.

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Inventions 2-18: claims 1-13, 15-39 and eventually claim 14. (all partially)

Mass tag reagents having the amino-reactive functionalities selected from the list below, their kits and uses for the characterization of molecules by mass spectroscopy and/or for molecule purification. Each class of amino-reactive functionalities listed below relates to a separate group of inventions, and claims 1-39 are partially to be considered within this group depending on the amino-reactive functionalities of the mass tag reagents. The separate classes of amino-reactive functionalities are: alkenyl sulphones, haloalkanes, maleimides, isocyanates, isothiocyanates, ketones, aldehydes, sulphonyl-halides, carboxylic-halides, anhydride esters, alkenes, N-hydroxysuccinimide esters, hydroxybenzotriazole esters, hydroxyazabenzotriazole esters, nitrophenyl esters, trichlorophenyl esters, pentafluorophenyl esters.

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Inventions 19-21: claims 1-39 (all partially, one or more of claims 6-10, 14, 22 and 25-29 are to be entirely excluded)

Mass tag reagents, their kits and uses for the characterization of molecules by mass spectroscopy and/or for molecule purification. A mass tag reagent according to these groups of inventions comprises a tertiary amine with two alkyl substituents that are: (i) separated, or (ii) linked together as to form a cyclic group with the nitrogen atom, or (iii) linked together as to form a cyclic group with the nitrogen atom and a further heteroatom. Each of these tertiary amine structures relates to a separate group of inventions, and claims 1-39 are partially to be considered within this group, or eventually excluded in their entirety, depending on the structure of the tertiary amine group.

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Inventions 22 and 23: claims 1-39 (all partially, eventually excluding claims 14 and 22 in their entirety)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Mass tag reagents, their kits and uses for the characterization of molecules by mass spectroscopy and/or for molecule purification. A mass tag reagent according to these groups of inventions comprises an alkylene or a phenylene linker between the tertiary amine and the amino-reactive functionality. The alkylene and the phenylene linkers relate to two separate groups of inventions. Claims 1-39 are partially to be considered within any of these groups, and claims 14 and 22 are eventually to be excluded in their entirety, depending on the structure of the linker.

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Invention 24: claims 1-18 (partially), 19

Method for the characterization of proteins and polypeptides by mass spectroscopy involving labelling the whole analyte and its fragments, which have been generated by means of a cleavage reagent, with mass tag reagents comprising a tertiary amine and an amino-reactive functionality. Claims 1-18 are partially to be considered within this group in so far as they only relate to the method of claim 19.

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Invention 25: claims 1-20 (partially), 21, 22, 36 and 37

Arrays of different mass tag reagents and method for the characterization of a plurality of molecules by mass spectroscopy involving labelling each molecule with a different mass tag reagent. Claims 1-20 are partially to be considered within this group in so far as they only concern a plurality of different mass tag reagents.

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Invention 26: claims 38 and 39

Kits for the purification of labelled analyte molecules comprising a mass tag reagent and a cation exchange resin.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003044864 A1	06-03-2003	WO 03031928 A2	17-04-2003
		CA 2462641 A1	10-04-2003
		EP 1446495 A2	18-08-2004
		WO 03029425 A2	10-04-2003
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